expanding chondrocytes, the chondrocytes were subcultured into 6 well culturing dishes at 4×10^5 cells/well. When cells grew into 80-90% confluent, the culture medium was changed for serum free medium or serum free medium with IL-1β (100pg/ml or 1ng/ml concentration). LIPUS treatment at 0, 7.5, 30, 120mW/cm^2 intensity was applied for 20 minutes. Total RNA was extracted immediately after 1 hour incubation. To elucidate the inhibitory effect of LIPUS on the articular degradation, the mRNA expression of MMP13 was analyzed by real-time PCR method. The condition of 0mW/cm^2 intensity without IL-1β was set as 1, and each of other conditions was shown as the relative amount. To compare any significant differences between control sample (LIPUS intensity 0mW/cm^2 without IL-1β or 0mW/cm^2 with IL-1β) and LIPUS-stimulated sample, the test results were statistically analyzed using the Student's t-test. The difference observed was considered to be significant when p value is lower than 0.05.

Results: LIPUS stimulation inhibited the mRNA expression of MMP13 induced by IL-1β of 100pg/ml concentration in intensity-dependent manner (0mW/cm^2: 4.67±1.60, 7.5mW/cm^2: 3.20±0.24, 30mW/cm^2: 2.06±0.55, 120mW/cm^2: 1.30±0.29). However, there were no significant differences when expression of MMP13 was induced by IL-1β of 1ng/ml concentration.

Conclusions: Our results indicate that LIPUS has a possibility to inhibit IL-1β induced mRNA expression of MMP13 in intensity-dependent manner on rat chondrocytes. Therefore, we may be able to use LIPUS as a daily useful modality to protect articular cartilage.

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THE MICRO-STRUCTURAL RESPONSE OF TENDON FASCICLES TO APPLIED STRAIN IS ALTERED WITH AGEING

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Purpose: The objectives of this study were to investigate the microstructural strain response and cell strain environment in the injury prone equine superficial digital flexor tendon (SDFT) and to determine if this response alters with increasing age. We hypothesise that the fibre level response to applied strain is heterogeneous and varies with ageing, with the result that cells within aged tendon are exposed to an altered strain environment.

Methods: Fascicles were dissected from the SDFT of 4 horses aged 4 to 6 years, and 4 horses aged 18 to 20 years (n=8 from each tendon). Fascicles were incubated in the collagen stain 5-dicholorotriazynl fluorescein, washed in PBS and secured in a tensile straining rig. Each fascicle was viewed under a confocal microscope using a x20 objective, and a grid was photobleached onto the fascicle (Fig. 1 A). Images of the fascicle were taken at 2% strain increments up to 10% (Fig. 1 B) and deformation of the grid at each strain increment quantified (Fig 1. C) by measuring changes in longitudinal strain (x + Δx), perpendicular strain (y + Δy), deviation from the vertical gridline (d1 + d2) and angle of the horizontal gridline (θx). Statistical significance was set at p<0.05 and is indicated by *. Data is displayed as mean ± SEM.

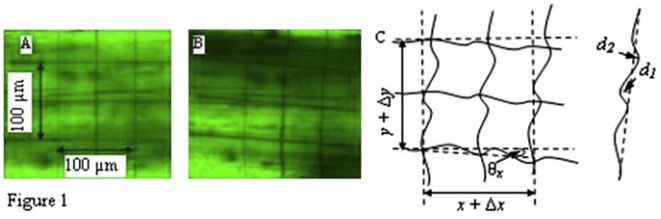

Figure 1

Results: Local longitudinal strains were heterogeneous, consistently smaller than applied strain, and did not alter with increasing age (Fig. 2).

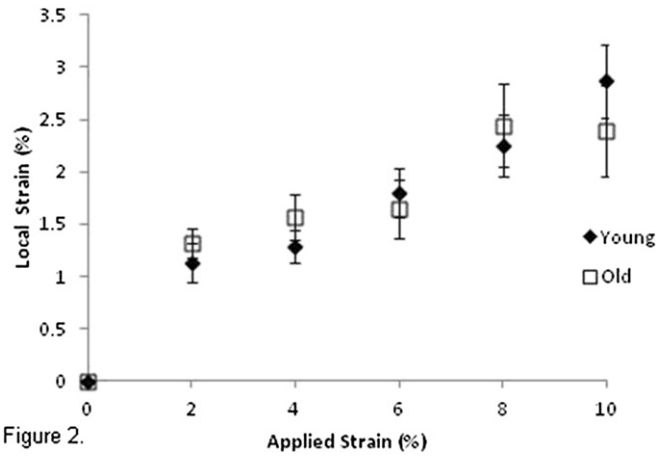

Figure 2.

Large compressive strains were measured perpendicular to the direction of applied strain; the magnitude of these strains decreased with increasing subject age (p≤0.016, Fig. 3).

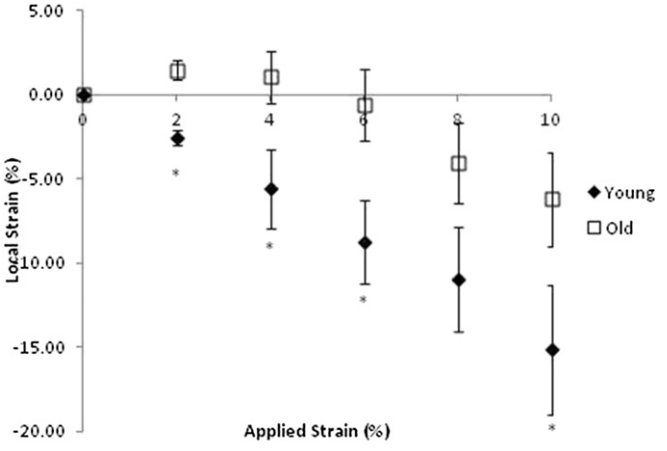

Figure 3.

Deviation from the vertical gridline increased with each strain increment (Fig. 4), indicating some sliding between adjacent fibres.

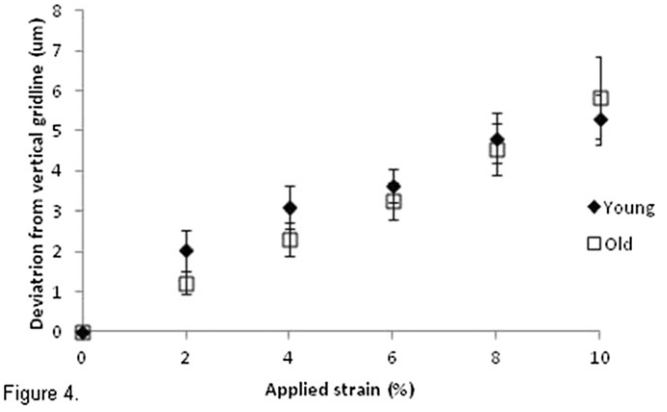

Figure 4.

The amount of fibre sliding did not change with increasing horse age. However, rotation of the horizontal gridline, which increased with each strain increment, did decrease significantly with age, at and above 8% strain (p≤0.033, Fig. 5).

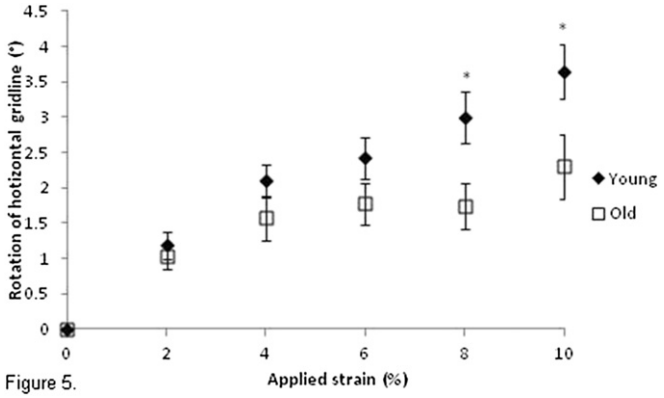

Figure 5.

Conclusions: The results support the hypothesis; strain distribution throughout the fascicles was complex and heterogeneous, with small local strains in the direction of applied strain, but large compressive strains perpendicular to the loading axis. Strain appears to be dissipated throughout the matrix by a combination of fibre extension and sliding. Rotation of the samples also occurred, suggesting a helical organisation to the fascicle structure; this may enable more efficient extension and recoil. These data suggest that, while the cells within tendon fascicles experience tensile strains much smaller than the applied strain, they are also subjected to shear and compressive strains. The decrease in compressive strain and rotation with increasing horse age may be due to changes in matrix organisation, and may significantly alter the local strains experienced by the cells. Decreased rotation with ageing also indicates some loss of the ability to recoil efficiently. These ageing changes may result in altered cell and mechanical responses to loading which could contribute to the increased risk of SDFT injury with ageing.

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TRANSPLANTATION OF ACHILLES TENDON TREATED WITH BMP-7 PROMOTED MENISCUS REGENERATION IN A RAT MASSIVE MENISCUS DEFECT MODEL

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Purpose: To preserve meniscus function, various meniscal substitutions such as meniscal allograft, collagen meniscus implant, and artificial materials have been tried in animal experiments or clinical studies. Transplantation of tendon is also one of possible treatments. BMP-7 is known to induce cartilage formation. Here we investigated the effect of BMP-7 on ectopic cartilage formation of tendon and outcome of transplantation of Achilles tendon treated with BMP-7 in a rat massive meniscus defect model.
Methods: (Study 1) Ectopic cartilage formation of tendon. After exposure of Achilles tendon in rats, 1μg of BMP-7 was injected into the tendon located anatomically. The tendon was evaluated histologically at 2, 3, and 4 weeks after the injection. (Study 2) Transplantation of Achilles tendon treated with BMP-7 for meniscal defect in a rat model. Untreated Achilles tendon was harvested and 1μg BMP-7 was injected. After anterior half of medial meniscus was resected, tendon treated with BMP-7 was transplanted into the meniscal defect. The rats were sacrificed at 4, 8 and 12 weeks after the surgery. As control groups, transplantation of Achilles tendon untreated with BMP-7 or only meniscectomy were performed. (Study 3) Analysis of cell kinetic. Achilles tendon derived from LacZ expressing rats were transplanted into meniscal defect of the wild rats.
Results: (Study 1) Injection of BMP-7 into Achilles tendon induced chondrocyte differentiation of tendon cells at two weeks. The number of chondrocytes evaluated with safranin-o staining, and type II collagen immunostaining, increased at 3 and 4 weeks. (Study 2) Macroscopically, transplantation of Achilles tendon irrespective of treatment of BMP-7 promoted meniscus regeneration. Microscopically, matrix of regenerated meniscus was already greater stained with safranin-o and type II collagen at 4 weeks and the meniscus became close to native meniscus at 12 weeks in the BMP-7 treated tendon transplantation group. Quantificational analyses demonstrated that the size of meniscus, histological score for regenerated meniscus, and histological score for articular cartilage were better in the BMP-7 treated tendon transplantation group than in other two groups ($p<0.05$; $n=6$). (Study 3) When LacZ expressing Achilles tendon was transplanted, LacZ positive cells were detected within the transplanted tendon tissue.
Conclusions: BMP-7 induced ectopic cartilage formation of tendon and transplantation of Achilles tendon treated with BMP-7 promoted meniscus regeneration and prevented cartilage degeneration in a rat massive meniscus defect model. Native cells in the Achilles tendon contributed to meniscal regeneration in this model.

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CHONDROGENIC, INFLAMMATORY AND FIBROTIC PROCESS IN PATHOLOGY OF IDIOPATHIC FROZEN SHOULDERS

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Purpose: To elucidate the chondrogenic, inflammatory, and fibrotic differentiation process in the pathogenesis of idiopathic frozen shoulders.
Methods: The protocols of this study were approved by both institutional review boards of Funabashi Orthopaedic Clinic and Tohoku University. From July 2007 to June 2009, we performed arthroscopic capsular release in 12 patients with idiopathic frozen shoulders, whose condition had failed to improve or had deteriorated after 6-months of conservative treatment. As a control group, 16 patients with rotator cuff tears without limited range of motion were selected. The difference of age distribution between these two groups was not statistically significant. Biopsy materials from the rotator interval capsule, middle glenohumeral ligament (MGHL), and inferior glenohumeral ligament (IGHL) were obtained during arthroscopic surgery. The number of cells was counted and the tissue elasticity of the samples was calculated by scanning acoustic microscopy (SAM). The amount of glycosaminoglycan content was assessed by alcian blue staining. Mast cells were stained with toluidine blue. Gene and protein expressions related to chodrogenesis, inflammation, and fibrosis were analyzed by quantitative polymerase chain reaction (qPCR), in situ hybridization (ISH), and immunohistochemistry (IHC). Furthermore, the total genes of the two groups were compared by DNA microarray analysis. SAM images of IGHL were compared with IHC of collagen type I and alcian blue staining and Pearson's product-moment correlation coefficient was calculated.
Results: The collagen bundles were dense with less space in idiopathic frozen shoulders, but the bundles were sparse and well-organized in shoulders with rotator cuff tears. Though the number of cells was significantly higher in idiopathic frozen shoulders, there were few cells expressing immunoreactivity of Ki-67 in both groups. The capsular tissue was significantly stiffer in idiopathic frozen shoulders by SAM. Staining intensity of alcian blue was significantly stronger in idiopathic frozen shoulders. Gene expressions related to fibrosis (COL1A1, COL3A1, PDGFB, α-SMA, and Substance P), inflammation (IL-1β), and chondrogenesis (ACAN, COL2A1, COLXA1, FOS, and FOSB) were significantly higher in idiopathic frozen shoulders by qPCR. Gene expression of TIMP-1 was significantly lower in idiopathic frozen shoulders. However, the expressions related to fibrosis (TGF-β, CTGF, PDGFA, HGF, MMP-1, 2, 3, and 9, TIMP-2 and 3), inflammation (TNF-α), and chondrogenesis (SOX9, ADAMTS4 and 5) were not changed in both groups. Fibroblast-like cells expressed ACAN signals in idiopathic frozen shoulders by ISH. Immunoreactivity of collagen type I and vimentin was stronger in idiopathic frozen shoulders. Immunoreactivity of α-SMA was not detected in fibroblast-like cells, but was detected in blood vessels in both groups. Comparing gray scale images of SAM, high sound speed area or low sound speed area did not correspond with any images in IHC of collagen type I and alcian blue